工學碩士 學位論文

주정폐수의 혐기성소화에 미치는 생물전기화학적인자

Bioelectrochemical factors on the anaerobic digestion of distillery wastewater

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Bioelectrochemical factors on the anaerobic digestion of distillery wastewater

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Abstract

생물전기화학기술은 혐기성소화조 내부에 산화전극과 환원전극 쌍을 설치하고, 외부전원을 이용하여 전극에 전압을 인가함으로서 전극들을 전기적으로 분극시키는 방법이다. 이러한 생물전기화학 혐기성소화는 혐기성소화조 내부에 높은 면적/부피 비로 설치할 때 혐기성소화조의 성능이 크게 향상된다. 그러나, 혐기성소화조 내부에 높은 면적/부피비로 설치된 전극들은 혐기성소화조의 교반이나 청소를 방해하고 초기시설비 및 유지관리비를 증가시킨다는 단점이 있다. 최근 전극사이의 벌크용액에 존재하는 전기활성을 가진 부유혐기성미생물이 메탄생성에 기여하는 바가 크며, 분극전극들 사이에 생성되는 정전기장이 전기활성미생물의 성장과 종간직접전자전달을 촉진할 수 있다는 것이 발견되었다. 그러나, 아직까지 정전기장의 세기가 생물전기화학 혐기성소화의 성능에 미치는 영향은 거의 연구되지 않았다. 따라서, 본 연구에서는 고분자 유전물질로 피복된 전극을 설치한 상향류식 혐기성반응조(Electric field upflow anaerobic bioelectrochemical anaerobic reactor, EF-UABE)에 인가전압을 단계적으로 증가시킴으로서 전기장의 세기가 주정폐수의 혐기성소화 효율에 미치는 영향을 연구를 하였다. 또한, 분극전극을 설치한 기존의 상향류식 생물전기화학 혐기성반응조(Conventional upflow anaerobic bioelectrochemical reactor, C-UABE)를 동일한 조건에서 운전하여 혐기성소화 효율을 비교하였다. 또한, 대조구로서 분극전극을 설치하지 않은 상향류식 혐기성반응조를 동일한 조건에서 운전하였다. EF-UABE에 0.5 V의 인가전압에서 운전을 시작하였으며, 정상상태의 메탄발생량은 386 mL/L.d 이었다. 이값은 C-UABE의 399 mL/L.d와 큰 차이가 없었으나, 대조구의 메탄발생량 101 mL/L.d 보다는 크게 높았

다. EF-UABE와 C-UABE에서의 메탄발생량의 차이가 크지 않은 것은 C-UABE의 메탄생성에 대한 분극전극표면의 기여도가 전극사이의 벌크용액에 존재하는 미생물의 기여도에 비하여 크지 않음을 나타낸다. EF-UABE 반응조에서는 인가전압의 증가에 따라 메탄발생량이 증가하였으며, 인가전압 5.0 V에서 456.4 mL/L.d로서 최대 값을 보였다. 그러나, 인가전압 10V에서는 메탄발생량이 393.9 mL/L.d로 약간 감소하였다. C-UABE 반응조의 메탄발생량은 인가전압 1.5V에서 432.9 mL/L.d 까지 증가하였으나 2.0V 이상에서는 인가전압의 증가에 따라 점차 감소하였다. 이 결과는 C-UABE 공정은 높은 전압을 인가할 경우 전극표면에서 일어나는 물의 전기분해 반응으로 인하여 부정적인 영향이 있다는 것을 나타낸다. 그러나, 전극의 표면을 유전물질로 절연한 EF-UABE 반응조에서는 물의 전기분해없이 높은 전압의 인가가 가능하며, 높은 인가전압에서는 전극 사이에 형성되는 정전기장이 더욱 커지게 된다. 또한, 높은 정전기장은 전기활성균의 전자전달반응을 더욱 촉진시켜 메탄생성율이 증가한다는 것을 나타낸다. EF-UABE 반응조에 설치한 전극은 고분자물질로 표면이 피복된 형태로서 C-UABE 반응조에 설치하는 전극에 비하여 저렴하게 제작과 설치가 가능하다. EF-UABE 반응조는 고농도 유기성 폐수처리를 위해 실용화가 용이한 생물전기화학 혐기성반응조이다.

KEY WORDS: Bioelectrochemical anaerobic digestion 생물전기화학, Anaerobic digestion 혐기성소화; Static electric field 정전기장; Methane production 메탄발생, Distillery wastewater 주정폐수

Chapter 1: Introduction

Anaerobic digestion is one of the traditional technologies for handling the organic matter and recovering clean energy as methane. Anaerobic digestion is generally considered to be a more sustainable and controllable way to deal with organic matter compared with other treatment routes such as landfill and composting (Wilkie et al., 2000). Anaerobic digestion has been widely used to manage sewage sludge, agricultural waste, brewery wastewater, food waste and other high concentration organic wastewater (Mata-Alvarez et al., 2000). However, anaerobic digestion still has some limitations including the low methane content (< 65%) and low organic degradation rate (sewage sludge <50%) as well as the instability of the anaerobic digestion process (Appels et al., 2008). These limitations are mainly caused by the slow hydrolysis rate and low growth rates of methanogenic archaea, which are relatively slow compared with acid producing bacteria (Song et al., 2016). Therefore, anaerobic digesters are generally operated at a lower organic loading rate or a longer HRT (> 20 days) and maintained at a stable temperature (35 ℃ or 55 ℃) (Feng et al., 2016a,b).

In recent, it has been revealed that the bioelectrochemical redox rates on the anode and cathode are improved by the polarized electric potential of the electrodes, and are less sensitive to the changes in the environmental conditions, such as influent pH and temperature (Pham et al., 2008). This means that the bioelectrochemical technology a potential approach to alleviate the limitations of anaerobic digestion and to improve the performance (Rozendal, et al. 2008). The bioelectrochemical technology can be combined with anaerobic digestion by installing a pair of polarized electrodes (anode and cathode) in the existing conventional anaerobic digester (Bajracharya, et al., 2016). In bioelectrochemical anaerobic digester, organic matter is oxidized on the anode surface into electrons, protons and carbon dioxide (Rozendal et al., 2008). The electrons are transferred from the anode to the cathode through an external circuit, and then the oxidized products are reduced on the cathode surface to form methane (Feng et al., 2016a,b). Compared with the conventional anaerobic digestion process, the bioelectrochemical anaerobic digestion for methane production shows several advantages, such as: i) more methane production and higher methane content in biogas; ii) requiring less thermal energy to maintain the temperature of the process; iii) the high concentration and dilution of organic waste material can be used as the process substrate; iv) the methanogenesis reaction is less sensitive to the environmental conditions, such as temperature, influent pH value, etc (Feng and Song, 2016b). However, this approach combining the bioelectrochemical technology with anaerobic digestion requires the electrode with highly biocompatible, conductive and

durable, and is effective in the field condition only when the area and volume ratio of the electrode is high (Song et al., 2016). The electrode with a high area and volume ratio can interfere with the agitation and cleaning work of the reactor, and increases the capital and operational costs (Feng et al., 2016a,b). In recent, the suspended anaerobic microorganisms in the bulk solution between the electrodes significantly contribute the methane production in the bioelectrochemical anaerobic digestion, and the electrostatic field can facilitates the direct interspecies electron transfer for the methane production (Lee et al., 2016). However, little studies have been done on the effect of electrostatic field intensity on the performance of bioelectrochemical anaerobic digestion.

In this thesis, the effect of electrostatic field on the performance of anaerobic digestion for distillery wastewater was studied in the upflow anaerobic bioelectrochemical anaerobic reactor installed with the electrode coated with a dielectric polymer (EF-UABE) by a step increasing the applied voltage. In addition, the performance in the conventional upflow anaerobic bioelectrochemical reactor with polarized bioelectrode (C-UABE) was studied at the same condition, and the performance was compared with the EF-UABE. An upflow anaerobic reactor without the electrode was also operated as a control.

Chapter 2: Literature Review

2.1 Distillery wastewater

2.1.1 Production of distillery wastewater

Alcohol is mainly produced by the fermentation of starchy substances, such as corn, wheat, sorghum and sweet potatoes, and some are produced from the sugar substances (Anderson, G., 1992). The main sources of distillery wastewater in the alcohol fermentation process are the distillation fermentation, as well as the washing water, the cooling water, and the soaking water for the raw materials. The distillery wastewater is commonly acidic (pH 3.5~4.5) and high in organic content and temperature (60~70 °C). The organic components in the distillery wastewater are sugar, organic acid, protein and cellulose. The values in COD, BOD_5, and SS of the distillery wastewater are about 40,000 mg/L, 20,000 mg/L, and 6,200 mg/L, respectively. This indicates that the distillery wastewater is one of the serious environmental pollution sources that threaten the living life.

2.1.2 Treatment status of distillery wastewater

The distillery wastewater is a high or medium strength organic wastewater containing various nutrients. The treatment of distillery wastewater to meet the discharge standards not only results in high pollution load, but also consumes a lot of infrastructure and operating costs. However, the organic matter contained in the distillery wastewater is a resource, as well as a pollutant. Therefore, for the distillery wastewater, an environmental countermeasure should be adopted, which is mainly based on comprehensive utilization and supplemented by pollution control. For the comprehensive utilization, the organic components in the wastewater are first separated, processed into feed or other by-products, and finally the wastewater is treated. This means that it is better to recover the materials contained in the wastewater and to utilize it as raw materials. This is a way that the enterprise can supply its social needs by the comprehensive utilization of the by-product, and achieve the economic benefits, environmental benefits, and social benefits.

The distillery wastewater has been commonly treated by the biological (biochemical) treatment process. The biological treatment of wastewater is divided into natural biological treatment (oxidation ponds, land treatment) and artificial biological treatment. Artificial biological treatment is generally divided into two major categories, aerobic and anaerobic. The biological treatment can be divided into suspension growth and attached growth, depending on the state of growth of microorganisms.

2.1.3 Aerobic biological treatment

Aerobic biological treatment is carried out by aerobic microorganisms under aerobic conditions. The aerobic process can be divided into activated sludge and biofilm process, depending on to the growth pattern of microorganisms in the treated structure.

Aerobic biological treatment is generally used to treat low strength organic wastewater. The domestic beer plants commonly use this method to treat the wastewater. In the activated sludge process, the COD influent concentration is 1,200 to 1,500 mg/L and the effluent concentration is 50 to 100mg/L, indicating that the COD removal efficiency is 92 to 96%. When the activated sludge is used to treat the medium strength distillery wastewater, the sludge bulking phenomena is observed. The sludge bulking is commonly caused by the high content of carbohydrates in the wastewater, lack of nutrients such as nitrogen, phosphorus, and iron, and the imbalance in carbon and nitrogen ratios. One of the solution for the sludge bulking is to mix domestic sewage with the brewery wastewater. Because the nitrogen content in domestic sewage is relatively high, the mixture of the two is more economical. Otherwise, technical measures such as adding nitrogen-containing chemical agents, adjusting the pH of wastewater, and improving the operating process conditions will be adopted.

In the biofilm process for brewery wastewater, influent COD is 1000~1500mg/L, and the effluent COD is 100~150mg/L. The problem that should be paid attention to when adopting biofilm process is that temperature. When the inlet water temperature is too high, it should be cooled in advance. It is easy to freeze in the winter in the north, so that the equipment cannot run normally. The pool is only suitable for areas where the average white temperature is not lower than 4°C. Although there are factory buildings, odors can be smelled from a distance.

In aerobic biological treatment, deep well aeration is recommended. It is an efficient wastewater biological treatment technology. It is a process developed by Royal Chemical Industry Corporation in 1968. The removal efficiencies of COD and BOD_5 were 84% and 96%. The features of deep well aeration are small footprint, high performance, and good impact resistance load performance. Although there is no precedent for using deep well aeration to treat brewery wastewater in China, but Bally, Ontario, Canada uses this technology to treat brewery wastewater. The removal efficiency of BOD_5 in the deep well aeration is 97.92%. It proves that deep well aeration is feasible for treating brewery wastewater.

For high strength organic wastewater, generally referred to as COD > 2000mg/L, BOD_5 > 1000mg/L wastewater, the use of aerobic biological treatment is economically unreasonable, it requires a lot of investment and land occupation, and the energy consumption is quite high. Therefore, an anaerobic biological treatment process is usually recommended.

Anaerobic treatment is a biological treatment of organic matter under anaerobic conditions. Anaerobic fermentation is not only a means to control wastewater pollution, but also produce methane gas.

Anaerobic treatment started in the 19th century, but the conventional anaerobic digestion requires higher temperature, longer hydraulic retention time, and lower treatment efficiency. The ever increasing wastewater pollution and the emergence of an energy crisis are being forced to seek new energy and to arouse research on this technology. In 1955, Schroefer proposed the anaerobic contact process, which marked the birth of modern anaerobic treatment technology. Since the late 1960s, anaerobic filter (AF), upflow anaerobic sludge blanket (UASB), and anaerobic fluidized bed (AEB) have emerged for high strength organic wastewater. The common feature of the processes, known as the second generation of anaerobic reactors, are a high volumetric loading rate and a short hydraulic retention time, so that the anaerobic reactor has a high efficiency. The second generation anaerobic reactor is completely suitable for the treatment of alcohol and brewery wastewater. This technology has broad prospects for applications in the sour wine industry. However, the COD effluent concentration from with the use of anaerobic treatment is still 500 ~ 1000mg/L, which still need the post-treatment to meet the standard discharge.

Although the treatment of the brewery industry wastewater is only suitable for biological treatment methods, there are many forms of treatment in both aerobic and anaerobic processes. In particular, the original treatment technology is increasingly perfect and new biological treatment technologies are emerging one after another. Various forms of biological treatment processes have been continuously enriched and coexisted. Most of the treatment technologies have its own advantages and applicable conditions. Therefore, the area where the factory is located, the area where the water treatment facilities are located, the scale and quality of the wastewater treatment, and the wastewater requirements of the environmental protection department, comprehensive analysis of factors such as energy consumption and secondary effects are needed in the selection of brewery wastewater treatment technologies. As many alternatives are selected as possible, and from repeated technical and economic arguments, it is possible to screen out technically feasible and economically reasonable processing technologies that are appropriate to the specific circumstances of the unit.

2.2 Anaerobic digestion

2.2.1 Fundamentals of anaerobic digestion

Anaerobic digestion of organic matter could be briefly defined as a biological conversion process of organic matters to methane and carbon dioxide under dissolved oxygen free condition. However, the anaerobic degradation pathways and the roles of microorganisms in the pathways are not generally clear yet. Anaerobic degradation process is simply described as a complex series-parallel reaction consisted of hydrolysis, acidogenesis, acetogenesis and methanogenesis. The hydrolysis step of particulate organic matter in the anaerobic degradation is generally known as a rate-limiting step that controls the overall degradation process and the rate could be affected by temperature, pH, hydrolytic enzymes and organic acids.

2.2.2 Microorganisms involved in anaerobic digestion

(1) Hydrolysis

Hydrolysis is an enzymatic reaction that is converted macromolecule organic material (carbohydrates, proteins, lipids) into monomer such as monosaccharides, amino acids, glycerol and long chain fatty acids (LCFAs). The hydrolytic enzymes are secreted by acidogenic microorganisms. According to Eastman and Ferguson (1981), particulate organic matters in the hydrolysis process are converted into soluble substrates, and the hydrolysis step is generally known as a rate-limiting step.

The hydrolysis rates of various organic materials are dependent upon the characteristics of substrates, anaerobic bacterial density, the hydrolytic enzymes, and the concentration of the final by-products as well as environmental conditions. The rate of hydrolysis and acidification as well as the VFA quality could be improved if the environment conditions such as dilution rate, organic loading rate, pH, and external electron acceptor were controlled properly.

(2) Acidogenesis

Acidogenesis is the reaction that converts the hydrolyzed monomers to short chain fatty acids (C2-C4), alcohol, carbon dioxide and hydrogen to obtain the carbon and energy sources for acidogens growth such as *Syntrobacter woliniiand and Syntrophomonas wolfei.* The spontaneous acidogenic reactions could occur thermodynamically if the hydrogen partial pressure is maintained at low level because it makes the free energy change for the reaction to a negative value. Here, the performance of acidogenic reaction depends on hydrogen partial pressure. It is well known that there is a symbiotic relationship between acetogens and methanogens. The low hydrogen partial pressure could be obtained by the hydrogenotrophic methanogens consuming hydrogen and carbon dioxide. The acetogenic bacteria convert ethanol, propionic acid, butyric acid to acetic acid and hydrogen.

2.2.3 Factors affecting anaerobic digestion

(1) HRT

HRT (Hydraulic retention time) is a major environmental factor in hydrolysis and acidification of anaerobic digestion. Generally, more VFA could be obtained at longer HRT, but the maximum production rate of VFA is obtained at shorter HRT. In an acidogenic digester operating at HRTs ranged from 6 to 15 hours, the VFA level increased until 12 hours of HRT, but the level of VFA was decreased at over 15 hours because of the methane produced by methanogenic bacteria. For particulate organic matter, the acidogenesis is not affected by the growth of microorganism, but the rate of hydrolysis mainly. Miron et al. (2000) reported that acidogenic condition is more favorable when HRT is less than 8 days because most of carbohydrate could be degraded. At 10 days of HRT, LCFAs could be oxidized partially, and methanogenic condition is favorable at over 10 days of HRT. Eastman and Ferguson(1981) studied on the hydrolysis and acidification of sewage sludge in short SRTs ranged from 9 to 72 hours at 35°C, and suggested that fat was not degraded in acidogenic phase, and the hydrolysis was a rate-limit step in acidogenic phase of particulate matter. Lilley et al. (1990) reported that the reaction of VFA formation was 1st order and 17 % of the influent COD could be converted into the VFA at 20°C and in less HRT than 10 days.

(2) Temperature

Acidogenic digester could be operated at two optimal temperature ranges which are the mesophilic condition of 30~38 ℃ and the thermophilic conditions of 55~60 ℃. When the acidogenic digester is operated at the mesophilic condition, the VFA loss to methane and the microbial decay rates are less than at the thermophilic acidogenesis, and the mesophilic acidogenesis is more cost effective because of the less heating energy requirement. The phase separation of the acidogenic stage at both the mesophilic and thermophilic conditions was more effective than psychrophilic condition, and the acid compositions were more stable at mesophilic range. Johannesburg (South Africa) is a temperate climate zone but the average temperature from 12 to 16°C, causing a decrease in VFA yields. Banister (1998) found that VFA yields (1% TS) decreased by 45% at 12°C compared to the yield observed at 22~28°C of acidogenic temperature at 6 days of HRT. Skalsky (1995) et al. reported that VFA production at 14°C was approximately 42% of 21°C.

(3) pH

pH is one of the important factors influencing the efficient anaerobic degradation of organic matter. Generally, methanogenic archaea are more sensitive to pH than the acidogenic bacteria. However, pH has so much influenced on the VFA formation or hydrolysis rate. In a general, two-phase anaerobic digestion, the optimum pH range for acidogenic digester is pH 5.0~6.0, and pH for methanogenic digester must be kept around pH 7.0. Valerie et al (1997) are reported that the hydrolysis percentage of protein was 5.7% at pH 5.0 and 82% at pH 9.0, that increased with pH. However, the hydrolysis rate of carbohydrate was 80% at pH 7 and less than 50% in other ranges of pH. Therefore, the optimum pH for overall substrate solubilization was ranged from 8.5 to 9.0, and the maximum VFA concentration was obtained at pH 8. The VFA formation rate was 35~40% at over pH 5.0, and the maximum conversion rate was appeared at pH 6.3. The amount of VFA produced at pH 3.6 was half of the pH 5.0. The values of pH in acidogenic reactor have an influence on the composition of VFA. They reported that the HAc in produced VFA was 83% at pH 7.0, but decreased to 60% and 33% at 6.5 and 6.3, respectively. The acidogenic system has some buffering capacity, and the pH could be maintained at 5.0~7.0 even if the system pH was not controlled by addition of acidic or alkaline material. However, the pH value depends on the acidogenic conditions including substrate characteristics.

(4) VFA Concentration and Hydrolysis Enzyme

VFA concentration is one of the most important parameter in anaerobic digestion. There are many studies on the inhibitory effect of VFA on the acidogenesis, and the pH drops in the anaerobic digester are mainly originated from the VFA. It is also believed that the intensity of VFA inhibition depends on pH determining the content of undissociated acid. Wang and Wang (1983) reported that the inhibitory effect of the undissociated acetic acid on the acidogenesis was higher than of the dissociated acetate ions. They argued that the inhibitory effect at the lower pH than 6.0 was mainly originated from the undissociated acid, but at over 6.0 of the pH, from the dissociated acid ion. In the hydrolysis of particulate materials, Denals et. al (1996) reported that the influent particulate COD in the first reactor, introducing a wastewater containing 90% of particulate COD, can be removed up to 50% within 4 hours with one tenth of returned biomass from the second reactor in two stage reactor system. This indicates that the slowly degradable particulate material could be hydrolyzed at higher ratio of biomass to substrate. Also, Llabres-Luengo et al (1988) proposed the hydrolysis kinetic model that the hydrolysis rate was proportional to the substrate volatile solids and the biomass and inversely to the VFA concentration. They concluded that the VFA inhibition governed the hydrolysis kinetics, but did not distinguish the effects of pH and VFA.

2.2.4 Pros and cons of anaerobic digestion

(1) The advantages of anaerobic wastewater treatment include:

Anaerobic wastewater treatment is the core technology of an integrated system that integrates environmental protection, energy recovery, and ecological benign circulation, with good environmental and economic benefits.

Anaerobic wastewater treatment technology is a very economical technology, and it is much cheaper than aerobic treatment in the cost of wastewater treatment, especially for wastewater with a medium or higher concentration (COD > 1500mg/L).

Anaerobic treatment not only requires little energy but also generates large amounts of energy.

Anaerobic wastewater treatment technology has high equipment load and small land occupation. The

volumetric loading rate of anaerobic reactor is much higher than the aerobic method, and the removal of organic matter per unit reactor volume is therefore much higher, especially using a new generation of high-speed anaerobic reactors.

The amount of excess sludge produced by the anaerobic process is much less than that of the aerobic process, and the excess sludge has good dewatering performance, and no dehydrating agent is used for concentration, so the remaining sludge treatment is easy.

The anaerobic method requires less nutrients. It is generally believed that if the biodegradable COD_{BD} is used as the calculation basis, the demand for nitrogen and phosphorus in the aerobic method is COD_{BD} : N : P = 100 : 5 : 1, while for the anaerobic method it is (350~500) : 5 : 1.

Anaerobic methods can handle high concentrations of organic wastewater.

Anaerobic bacterial strains can retain their biological activity and good sedimentation performance for at least one year when the supply of wastewater and nutrients is terminated.

The anaerobic system is flexible in scale, simple in equipment, easy to manufacture, and does not require expensive equipment.

(2) Insufficiency of anaerobic treatment:

Due to the sensitivity of methanogens and the extreme instability of the obligate anaerobic microbial enzyme system to oxygen, the anaerobic microorganisms in the reactor show less change to the environment than the aerobic system. Therefore, the anaerobic system has a long start-up time, which seriously affects the application of anaerobic processes in wastewater treatment.

Although the organic loading rate is high in the anaerobic digestion, its effluent quality is worse than the aerobic treatment. This means that the post-treatment is required to achieve a higher discharge standard.

Anaerobic microorganisms are more sensitive to toxic substances. Therefore, the poor understanding of

the properties of toxic wastewater or improper operation leads to deterioration of the operating conditions, and causes the reactor to “acidify”.

2.3 Bioelectrochemical anaerobic digestion

2.3.1 Fundamentals of bioelectrochemical anaerobic digestion

Bioelectrochemical technology (BET) can be coupled with anaerobic digestion by installing anode and cathode inside an existing conventional anaerobic digester, and maintaining a small potential difference between the anode and cathode (Fig. 2.1) (Song et al., 2016). One of the methane production mechanisms in the bioelectrochemical anaerobic digestion is described by followings; i) organic matter is oxidized on the anode surface into electrons, protons and carbon dioxide, ii) the electrons are transferred from the anode to the cathode through an external circuit via the applied voltage, and then the oxidized products are reduced on the cathode surface to form methane. The reactions on the surface of anode and cathode are explained by equation 2.1 and equation 2.2, respectively (Song et al., 2016; Cheng et al., 2009).

$$CH_3COOH + 2H_2O \rightarrow 2CO_2 + 8H^+ + 8e^-,\ E_{pa} = -0.486V\ (vs.\ Ag/AgCl) \quad (2.1)$$

$$CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2H_2O,\ E_{pc} = -0.445V\ (vs.\ Ag/AgCl) \quad (2.2)$$

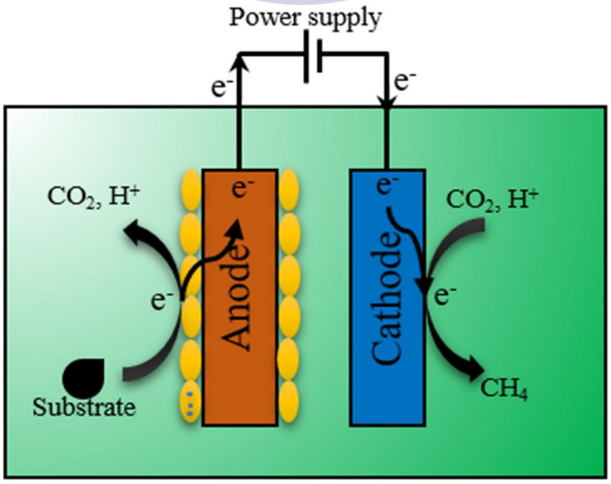


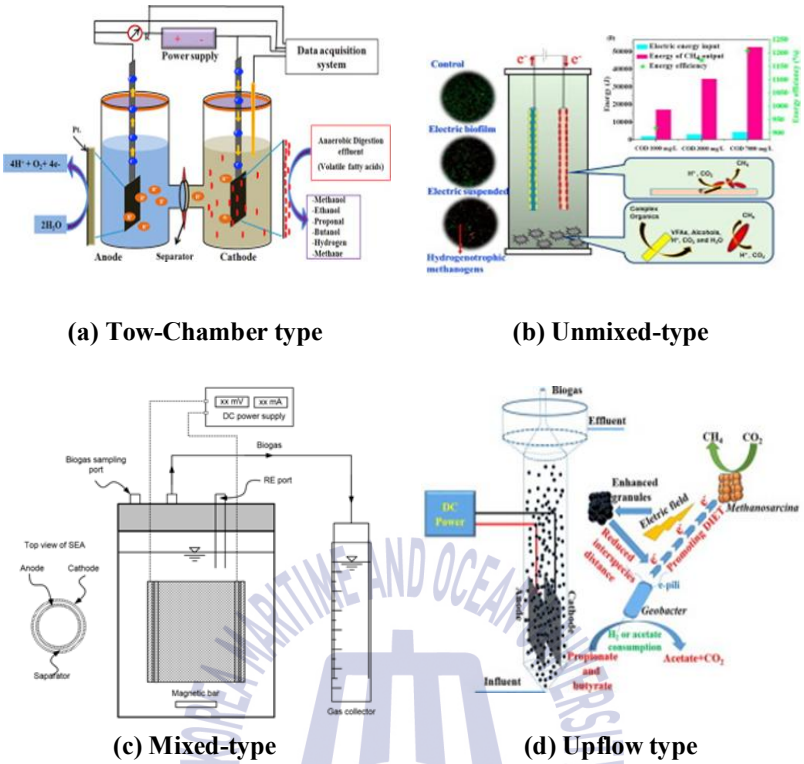
Fig. 2.1. Schematics of bioelectrochemical anaerobic digestion.

In recent studies, the methane production at the electrode surface of a bioelectrochemical anaerobic

digester is reported to be less than 20% (Zhao et al., 2015; Shen et al., 016). It is also mainly attributed to the enhanced direct interspecies electron transfer (DIET) by the enrichment of electroactive bacteria (Kato, 2015). When a redox compound is present in an anaerobic digester, it was observed that the electroactive bacteria reduce the redox compound to transfer the electron, and then the methanogenic bacteria use the electron from the compound to produce methane (Lovley, 2011; Marsili et al., 2008; Richter & Gescher, 2014; Shen et al., 2016; Shrestha et al., 2014). It is known that when the methanogenic bacteria are in close proximity to the electroactive bacteria via either the anode and cathode in an anaerobic reactor coupled with bioelectrochemical devices, or through a conductive material in the anaerobic reactor, the methanogenic bacteria produce methane from the reduction of carbon dioxide using the electrons transferred directly from electroactive bacteria (Dube & Guiot, 2015; Rotaru et al., 2014a; Shen et al., 2016; Shrestha et al., 2014). These types of electron transfer pathways for methane production are referred to as direct interspecies electron transfer (DIET). However, the electron transfer pathway for methane production in the bioelectrochemical anaerobic digester is sparsely studied.

2.3.2 Status of bioelectrochemical anaerobic digestion

Bioelectrochemical anaerobic digestion is a new and promising approach for methane production from wastewater, organic matter and other renewable resources (Kadier et al., 2014). The bioelectrochemical anaerobic digester is easily constructed by installing anode and cathode inside an existing conventional anaerobic digester, and applying a little electric energy (Song et al., 2016). The process performance in methane production and organic removal is considerably enhanced, especially the methane content in biogas is in the range of 70% - 90% (Xafenias & Mapelli, 2014; Chen et al., 2015; Song et al., 2016; Zhao et al., 2016), which is much higher than a conventional anaerobic digester, increasing the possibility of direct application as an energy resource. However, until now, bioelectrochemical anaerobic digestion was mostly studied in small scale batch reactor using synthetic wastewater at mesophilic condition. (Wang et al., 2009; Cheng et al, 2009; Sasaki et al., 2011; Gajaraj et al., 2017). Various types of bioelectrochemical anaerobic digester like two-chambers, unmixed-type, mixed-type and upflow type shown in Fig 2.2, were used (Kondaveeti & min, 2015, Li et al., 2016; Feng & Song, 2016a; Wang et al., 2017), but and it has been rarely studied as a continuous system with complex substrates, such as sewage sludge and distillery wastewater. Therefore, bioelectrochemical anaerobic digestion requires more studies on the detailed process for the treatment of complex organic matters.



(a) Tow-Chamber type **(b) Unmixed-type**

(c) Mixed-type **(d) Upflow type**

Fig. 2.2. Reactor types of bioelectrochemical anaerobic digestion.

2.4 Limitations of bioelectrochemical anaerobic digestion

(1) Electrode

The bioelectrochemical electrodes, including anode and cathode, have been studied earlier, however, these studies were primarily focused on microbial fuel cells (Song et al., 2015ab; Nan et al., 2011). The available information on bioelectrochemical electrodes is still limited. The general considerations for bioelectrochemical anode are as follows: i) high conductivity, ii) affinity for microorganisms growing, (iii) a porous material having a large specific surface area for microorganisms attaching, (iv) chemical and biological stability and durability, (v) the shape of the electrode should be easily manufactured, (vi) inexpensive materials, (vii) no clogging for the overgrowth of microorganisms, (viii) easy scale-up for bioelectrochemical anaerobic digestion (Liang et al. 2011; Song et al., 2015a). The bioelectrochemical

cathode should also have similar characteristics with its electrode equivalent. In theory, the bioelectrochemical cathode acts as an electron acceptor, as it uses the electrons transferred from anode to reduce carbon dioxide to methane. For better efficiency, the catalyst of the cathode plays a vital role in accelerating the reaction rate of methane production from carbon dioxide. Therefore, the electrochemical properties of bioelectrochemical cathode plays an important role in determining the reaction rate. However, available information on the cathode materials is also not adequate. In order to transfer the electrons to the surface of bioelectrochemical cathode smoothly, a highly conductive material is used. The bioelectrochemical cathode should have a wide specific surface area so that the reduction reaction proceeds efficiently. A suitable catalyst on the cathode can increase the efficiency of methane production by reducing carbon dioxide.

Table 2.1 Status of bioelectrochemical anaerobic digestion according to few representative studies

Substrate	Volume	Electrode materials	Voltage	Methane Production	Reference
Activated sludge	0.8 L	Reticulated vitreous carbon	0.3~0.6v	1.65 ml/L	Gajaraj et al., 2017
Artificial wastewater	0.49 L	Carbon-felt	Cathode potential: -0.85-1.15 V (vs. Ag/AgCl)	0.27 L.d	Jiang et al., 2013
Synthetic wastewater	1 L	Graphite	1.0-1.5 V	1.2 L/L.d	Li et al., 2016
Artificial wastewater	0.3 L	Ti/Ru alloy mesh plate	1.4~1.8V	0.43-0.53 L/L	Guo, et al., 2013
Sewage sludge	4.0 L	Carbon fiber fabric	Cathode potential: 0.8 V (vs. Ag/AgCl)	2.35 L/L.d	Sasaki et al., 2013
F-T wastewater	4.8 L	Graphite felt (GF)	1.5 V	2.31 L/L.d	Wang et al., 2017
Glucose & acetate	1 L	Graphite	0.5-1.0 V	0.94-0.99 L/L.d	Zhao et al., 2014

Until now, most carbon based materials generally meet the requirements of the bioelectrochemical anode and cathode. Materials such as carbon paper, carbon plate, carbon cloth, graphite rod graphite granule, reticulated vitrified carbon, and multiwall carbon nanotube have been widely used as bioelectrochemical electrodes (Table 2.1) (Song et al., 2015ab; Feng & Song, 2016a,b). However, a more efficient and durable electrode material is still required to achieve high-rate bioelectrochemical methane production.

(2) Applied voltage

In a bioelectrochemical anaerobic digester, the potential difference between anode and cathode is one of the important factors for efficient operation. Organic matter is oxidized by electroactive bacteria, which adhering onto the surface of anode, and produce protons, carbon dioxide, and electrons. The electrons are transferred to the cathode, where carbon dioxide, and protons are reduced into methane by applying a small voltage with a DC power supply (Liang et al., 2011; Liu et al., 2012). Therefore, the potential difference between anode and cathode is the driving force for the electrons, which can be affected by applied voltage, internal resistance of electrode, and other external conditions (Rader & Logan, 2010; Nam et al., 2011). The bioelectrochemical reaction does not occur when the potential difference is too low (<0.2 V), but the electrolysis of water occurs if the potential difference is too large (1.48 V, theoretical value: 1.23 V) (Logan, 2008). Theoretically, anode potential should be more positive than E_{pa} (-486 mV vs. Ag/AgCl) and cathode potential should be more negative than E_{pc} (-445 mV vs. Ag/AgCl) (Hamelers et al., 2010). According to a previous study (Wang et al., 2009), maximum amount of hydrogen gas was generated when applied voltage was in the range of 0.5- 0.9 V, but methane gas was generated when applied voltage was in the range of 0.3-0.6 V in a MEC in a previous study. However, the applied voltage for bioelectrochemical anaerobic digestion is still sparsely studied and remains unclear.

(3) Others

Recently, it was proven that bioelectrochemical systems, such as microbial fuel cells (MFCs) and microbial electrolysis cells (MECs), are less sensitive to external environment conditions, such as influent pH and temperature (Larrosa-Guerrero et al., 2010; Heidrich et al., 2014). In a previous study, the biogas production rate increased by 30% at a pH of 5.8 than that at 7.0 pH in a bioelectrochemical reactor (Hu et al., 2008). The methane yield in a bioelectrochemical anaerobic digester at 10℃ was 5.3 - 6.6 times higher than a control digester (without applied voltage and electrodes) operated at 10 ℃, and equivalent to the yield of a control digester operated at mesophilic condition (35 ℃) (Liu, et al., 2016). However, it is difficult to simply define that a bioelectrochemical anaerobic digester is not affected by the external environment. In theory, keeping in tune with a conventional anaerobic digester, the performance of a bioelectrochemical anaerobic digester is affected by influent characteristics, such as organic matter, organic loading rate, HRT, pH, temperature etc. It is also reported that the performance of bioelectrochemical reactors were slightly reduced by decreasing pH and temperature, but still higher than the control reactor at the same operation condition (Yuan et al., 2011; Feng et al., 2016a,b). It implies that

the electroactive bacteria adhering onto the surface of electrode can be affected by external environment, but not as much as a conventional anaerobic digester.

Chapter 3: Materials and methods

3.1. Anaerobic upflow reactors and operation

The upflow anaerobic reactor used in this experiment was prepared using a cylindrical acrylic resin (effective volume 1.0 L, inner diameter 7 cm). A flanged cover plate was used for each reactor to ensure that the upper end of the reactor was airtight in each case. An inlet valve that flows into the wastewater is installed on the bottom wall of the reactor, and an outlet valve is installed on the wall below the headspace of the reactor. The outlet valve is also connected to the U-tube to prevent biogas from leaking from the headspace of the reactor. The upper portion of the reactor was sealed with an acrylic cover and three ports were placed on the cover for biogas sampling, reference electrode retention and biogas venting. The biogas sampling port was covered with a butyl rubber stopper, and the reference electrode holding port was sealed by connecting a gas tight tube immersed in the digestion solution to the bottom side of the cap plate. The biogas vent is connected to a floating gas collector that is filled with a saturated salt solution and acidified to reduce biogas dissolution (Walker et al., 2009). An upflow reactor was used as a control reactor in the experiment, and the other two upflow reactors were used as EF-UABE and C-UABE reactors by vertically mounting the electrodes. The potential difference between the anode and cathode in the EF-UABE and C-UABE reactors was controlled by using a direct current (DC) power source (OPM series, ODA Technologies Co., Incheon, Korea). The prepared upflow reactor was installed in a temperature controlled chamber (35 ± 2 ° C). The upflow reactor was inoculated with anaerobic sludge collected from a sewage treatment plant (1.0 L, collected from the S sewage treatment plant, Busan, Korea). Distillation wastewater (collected from the ethanol industry, MH ethanol, Masan, Korea) whose pH was adjusted to 6.5 was continuously added to the reactor using a peristaltic pump. Table 1 shows the characteristics of distillation wastewater and seed sludge.

3.2. Electrode fabrication

In this experiment, two sets of electrodes were fabricated. For the anode in the EF-UABE reactor, a stainless steel mesh (20 * 18 cm), multi-walled carbon nanotubes (MWCNT, Carbon Nanomaterials Technology Co., Ltd., Korea) and exfoliated graphite (EG, Hyundai Coma Industry), Inc. were used. ., Korea), polyethyleneimine (PEI, Acros Organics) and ethanol (SK chemicals, Korea). The MWCNTs were immersed in concentrated nitric acid for 24 hours and then rinsed with running tap water to remove

impurities and improve surface hydrophilicity. The EG was stripped by microwave irradiation for 10 seconds and then reduced in a hydrazine solution as previously studied (Kim et al., 2015). Then, MWCNT, EG and PEI were mixed and stirred at 1:1:1 and sonicated (Power Sonic 420) for 1 hour to ensure thorough mixing of the materials. The mixture paste was screen printed on the surface of the stainless steel mesh to form a stent layer. For the cathode of the reactor, stainless steel rods and titanium wires were used, and the titanium wires were uniformly wound around the stainless steel rods, leaving 35 cm as a connection power source. After the production is completed, the coating electrode (SEAL COAT® CLEAR URETHANE COATING, 11 WT OZ) is used for insulation treatment. The electrodes in the C-UABE reactor are not insulated.

Table 3.1 Characteristics of seed sludge and distillery wastewater

Parameters	Granular sludge	Anaerobic sludge	Distillery wastewater
pH	7.34	6.52	3.6 ± 0.2
Alkalinity (mg/L as CaCO3)	-	683	-
VFAs (mg/L as COD)	-	364	-
TS (g/L)	-	16.2	33.1 ± 6.9
VS (g/L)	-	8.5	27.9 ± 5.9
TCOD (g/L)	-	20.6	30.2 ± 5.6
SCOD (g/L)	-	1.9	16.6 ± 6.1
Sulfate (g/L)	-	-	1.6 ± 0.2
Total nitrogen (g/L)	-	-	0.62 ± 0.07

3.3 Analysis and calculation

The biogas composition was analyzed by a gas chromatography (Series 580, GawMac Instrument Co., PA, USA) connected with a Porapak Q column (6 ft * 1/8th" SS) and thermal conductivity detector. The production of biogas was monitored from the gas collector, and converted to standard temperature and pressure by the correction of water vapor pressure at 35℃ using Eq.(1) (Feng and Song, 2016b).

$$V_{biogas}(L, atSTP) = V_{biogas}(at\ T) \times \frac{273}{273 + T} \times \frac{760 - W}{760} \quad (1)$$

Where, T is the temperature of the operation room and W is the water vapor pressure at 35°C (mm Hg). The biogas production (V_{biogas}) is the total biogas production at each monitoring time interval measured from the gas collector. Then, the methane production rate was calculated from the total biogas production and their methane contents, and divided by the total effective volume of the digester (1.0 L). The pH of the effluent wastewater was daily analyzed with a pH meter (Orion Model 370), and the COD was measured according to the standard method (2005). The methane yield was estimated as the methane production per 1g of COD removed. The total alkalinity (as $CaCO_3$) and volatile fatty acids (VFAs) concentration was measured with a titration method (Anderson and Yang, 1992), and the VFA composition was analyzed with an Aminex HPX-87H column and UV (ultraviolet) detector equipped on a high performance liquid chromatography instrument. The electric current between anode and cathode was monitored with a digital multimeter at steady state (DMM:Ni cDAQ-9174, National Instruments).

In addition, cyclic voltammetry (CV) for the bulk solution (100mL) was also conducted in the potential range between -1.0 and 1.0 V (vs. Ag/AgCl reference electrode) with a 10mV s-1 scan rate using the electrochemical instrument (ZIVE SP1, Won-A Tech, South Korea). For the CV test, small pieces of stainless mesh (1 cm×1 cm) were used as the working and counter electrodes. The peak currents for the oxidation and reduction and the potential values at peak current were obtained from CV data using the 'SMART Manager' analysis software.

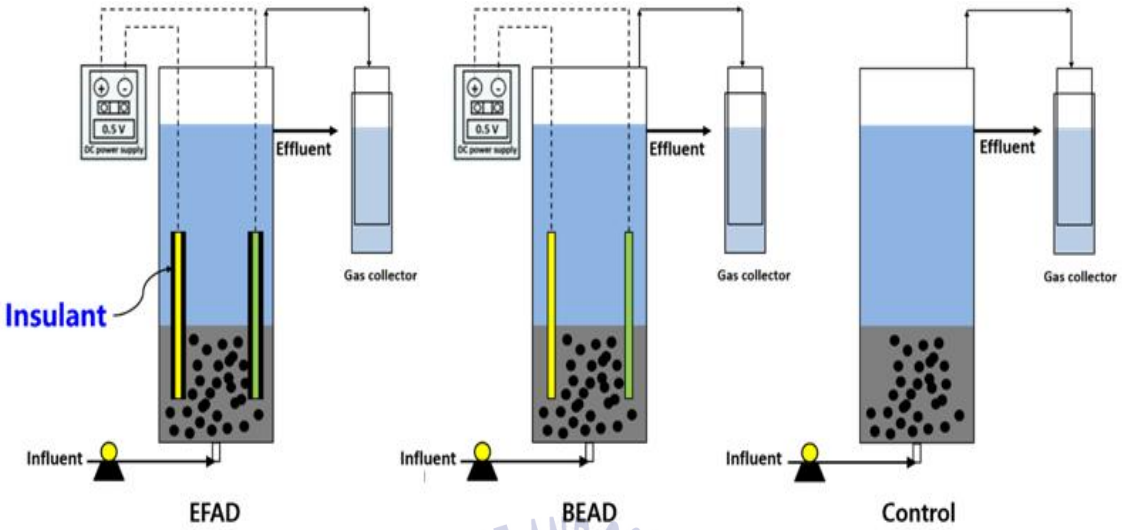


Fig. 3.1. Schematic diagram of the EF-UABE and C-UABE reactor and the control reactor

Chapter 4: Results and discussion

4.1 State variables of upflow anaerobic reactors

The changes in pH, alkalinity, and VFA give the insight into the stability of anaerobic digestion (Liu et al., 2012). The proper pH range for the growth of methanogenic microorganisms can be maintained by supplying sufficient alkalinity (Gulhane et al., 2016). Fig. 4.1 shows that the changes in the pH for the EF-UABE and C-UABE reactor and the control. When the voltage of 0.5 V was applied for the start-up, the pH in the EF-UABE reactor was initially dropped, and gradually recovered to 7.52, which was similar to 7.58 in the C-UABE reactor. However, the pH in the control was 7.03 (Table 4.1). When the voltage was increased step-by-step to 5.0 V, the pH of the EF-UABE reactor was considerably stable at around 7.50. In the C-UABE reactor, the pH was maintained until the applied voltage increased to 2.0V, but decreased to 7.17 at 5.0 V. At 10 V of the applied voltage, the pH in the EF-UABE was 7.63, which was higher than 7.24 in the C-UABE. In conventional anaerobic digestion, the optimal pH is ranged from 6.8 to 7.8 (Yang et al., 2015). It seems that the pH in both upflow anaerobic reactors were in the proper range, but the buffer capacity of the EF-UABE was slightly higher than the C-UABE reactors or the control.

Fig. 4.2 shows that the changes in the alkalinity for the EF-UABE and C-UABE reactor and the control. After the start-up of the upflow anaerobic reactors at 0.5 V of the applied voltage, the alkalinity of the EF-UABE and the C-UABE were gradually decreased, and stabilized at 4,836 mg/L as $CaCO_3$ and 5,180 mg/L $CaCO_3$, respectively, which was considerably lower than the 5,579 mg/L as $CaCO_3$ in the control. The high alkalinity in the control seems that the acidogenic fermentation was not active compared to the hydrolysis of the nitrogenous compounds that produce the ammonium bicarbonate alkalinity. At 5.0 V of the applied voltage, the alkalinity of the EF-UABE and the C-UABE reactor were stabilized at 4,923 mg/L as $CaCO_3$, and 4,580 mg/L as $CaCO_3$ (Table 4.1). At 10 V of the applied voltage, the alkalinity of the EF-UABE reactor was increased to 5,803 mg/L as $CaCO_3$, but there was no significant changes in the alkalinity of the C-UABE reactor. In conventional anaerobic digestion, the optimal range of alkalinity is in the range of 4,000 mg/L as $CaCO_3$ to 6,000 mg/L as $CaCO_3$ (Kardos et al., 2011; Song et al., 2004). In general, alkalinity in anaerobic reactor can be produced by the degradation of nitrogenous organic compounds and the reduction of carbon dioxide and sulfate (Song et al., 2004; Song et al., 2016). This means that more hydrolysis and methane production is followed by higher alkalinity. The high alkalinity

in the EF-UABE demonstrates that the hydrolysis and methanogenesis was more active compared to the C-UABE. It is well known that the methanogenesis is improved by the DIET pathway for the methane production. This is consistent with the methane production and COD removal in the EF-UABE, which were higher than the C-UABE reactor when the applied voltage was in the range of 2.0 V to 5.0 V (Table 4.1).

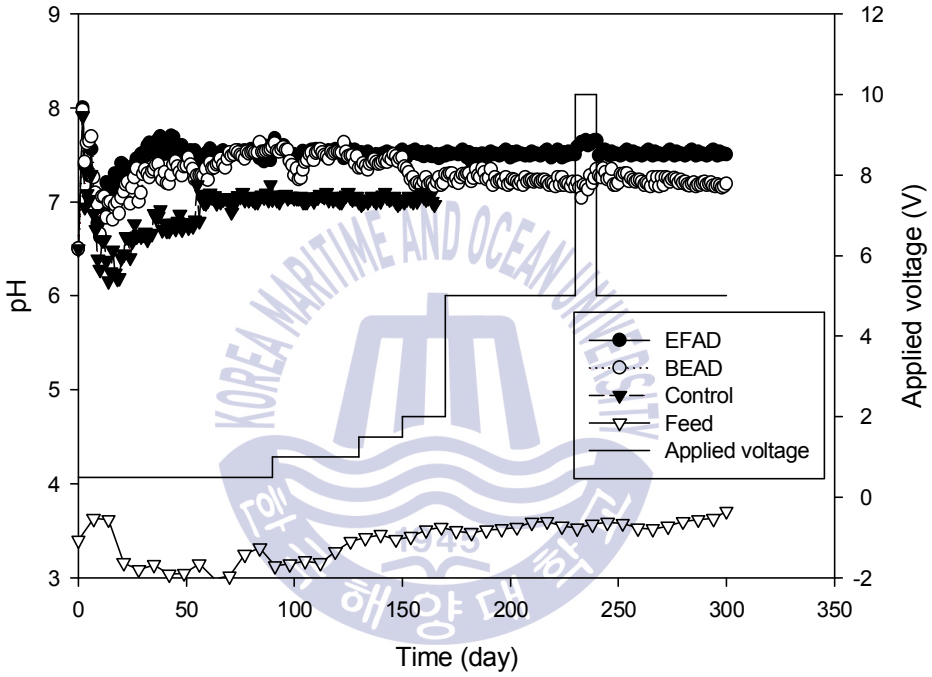


Fig. 4.1. Changes of the pH for the influent and effluent in the upflow reactors

Table 4.1 State variables in the EF-UABE, C-UABE and control reactor

Reactors	Applied voltage (V)	pH	Alkalinity (mg/L as $CaCO_3$)	VFA (mg/L as COD)
EF-UABE	0.5	7.51±0.01	4836.6±388	385.4±21.3
	1.0	7.52±0.03	5027.4±137	348.7±29.6
	1.5	7.51±0.04	5024.6±110	356.7±24.1
	2.0	7.49±0.02	5113.6±53	320.5±37.9
	5.0	7.50±0.05	4923.4±89	298.1±32.1
	10	7.63±0.14	5803.6±231	452.3±43.8
	5.0re	7.51±0.02	5472.2±67	
C-UABE	0.5	7.55±0.02	5180.4±293	366.7±25.4
	1.0	7.45±0.05	4569.6±173	324.1±32.5
	1.5	7.47±0.03	4479.4±117	398.2±39.3
	2.0	7.23±0.12	4708.4±123	520.4±32.1
	5.0	7.17±0.05	4580.8±180	787.1±35.0
	10	7.24±0.27	4755±351	1165.4±42.8
	5.0re	7.17±0.14	4594.2±99	
Control	\	7.03±0.02	5579.6±70	1240.1±47.3

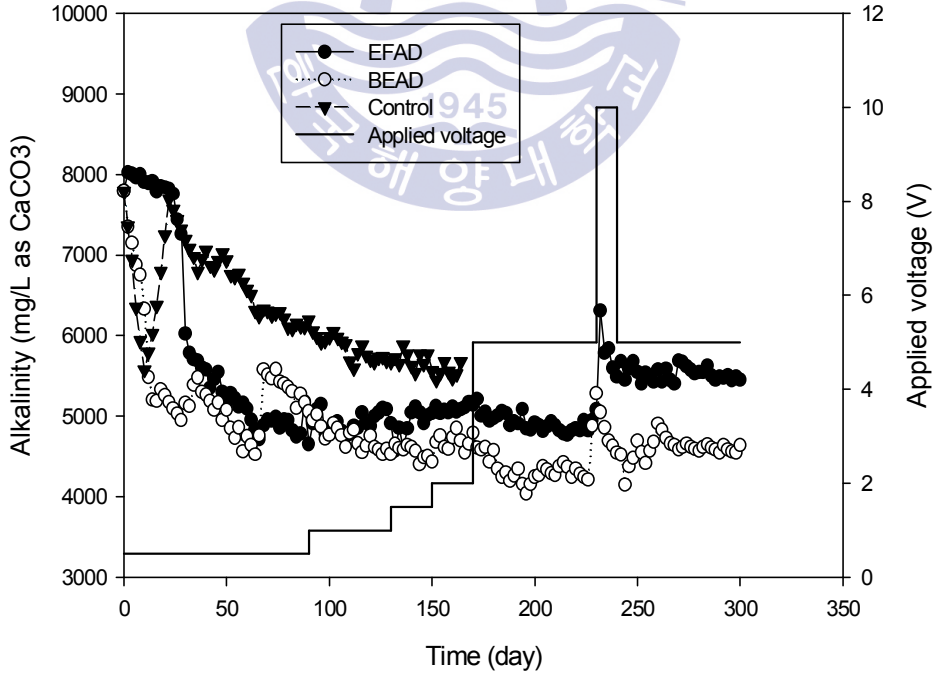


Fig. 4.2. Changes of the alkalinity for influent and effluent in the upflow reactors

The anaerobic digestion process of organic matter is reflected by the concentration of VFAs and their distributions (Song et al., 2004; Lee et al., 2015). Fig. 4.3 shows the concentration of VFAs and their distributions at steady state. At 0.5 V of the applied voltage, the VFA for the EF-UABE reactor was 385 mg COD/L, which was similar to 366 mg COD/L for the C-UABE reactor (Fig. 4.3). When the voltage was increased to 5.0 V, the VFA in the EF-UABE reactor was 298(Fig. 4.3a) mg COD/L, which was much lower than 787(Fig. 4.3b) mg COD/L of the C-UABE reactor. However, in the control reactor, the VFA level was as high as 1,240 mg COD/L. When the applied voltage was stepped-up to 10.0 V, the VFA level in the EF-UABE reactor was slightly increased to 452(Fig. 4.3a) mg COD/L, but it was considerably increased to 1,165 mg COD/L in the C-UABE reactor (Fig. 4.3). The low VFA level indicates that anaerobic digestion process is well balanced between the acidogenesis and methanogenesis (Song et al., 2004; Hartmann & Ahring, 2005). The accumulation of VFAs is generally involved in the unstable anaerobic digestion. This indicates that the anaerobic degradation of the substrate were well balanced in both EF-UABE and C-UABE reactors when the applied voltage was less than 5.0 V. In particular, the VFA levels in the EF-UABE and C-UABE reactors were always lower than the control. The VFAs were consisted of formate, acetate, propionate and butyrate in the EF-UABE and C-UABE reactors when the applied voltage was less than 5.0 V, but a long chain-fatty acid of caproate was observed in the control reactor (Fig. 5). Generally, long chain-fatty acid inhibits the methanogenic activity in anaerobic digestion (Hanaki et al., 1981; Koster & Cramer, 1987). As shown in Fig. 4.3, the formate concentration of EF-UABE and C-UABE reactors were always lower than the control reactor. It means that the hydrogen/formate are more accumulated in the control, compared to the EF-UABE and C-UABE reactors. Commonly, the hydrogen and formate are produced during the acidogenic fermentation when the $NADH/NAD^+$ ratio is high in the bacterial cells. The hydrogen/formate are the intermediates transferring the electrons to carbon dioxide to produce methane (Mir et al., 2016; Shrestha et al., 2014). This electron transfer pathway via intermediates for methane production is called as indirect interspecies electron transfer (IIET). It seems that the DIET pathway is contributed more to the methane production in the EF-UABE and C-UABE reactors, compared to the control.

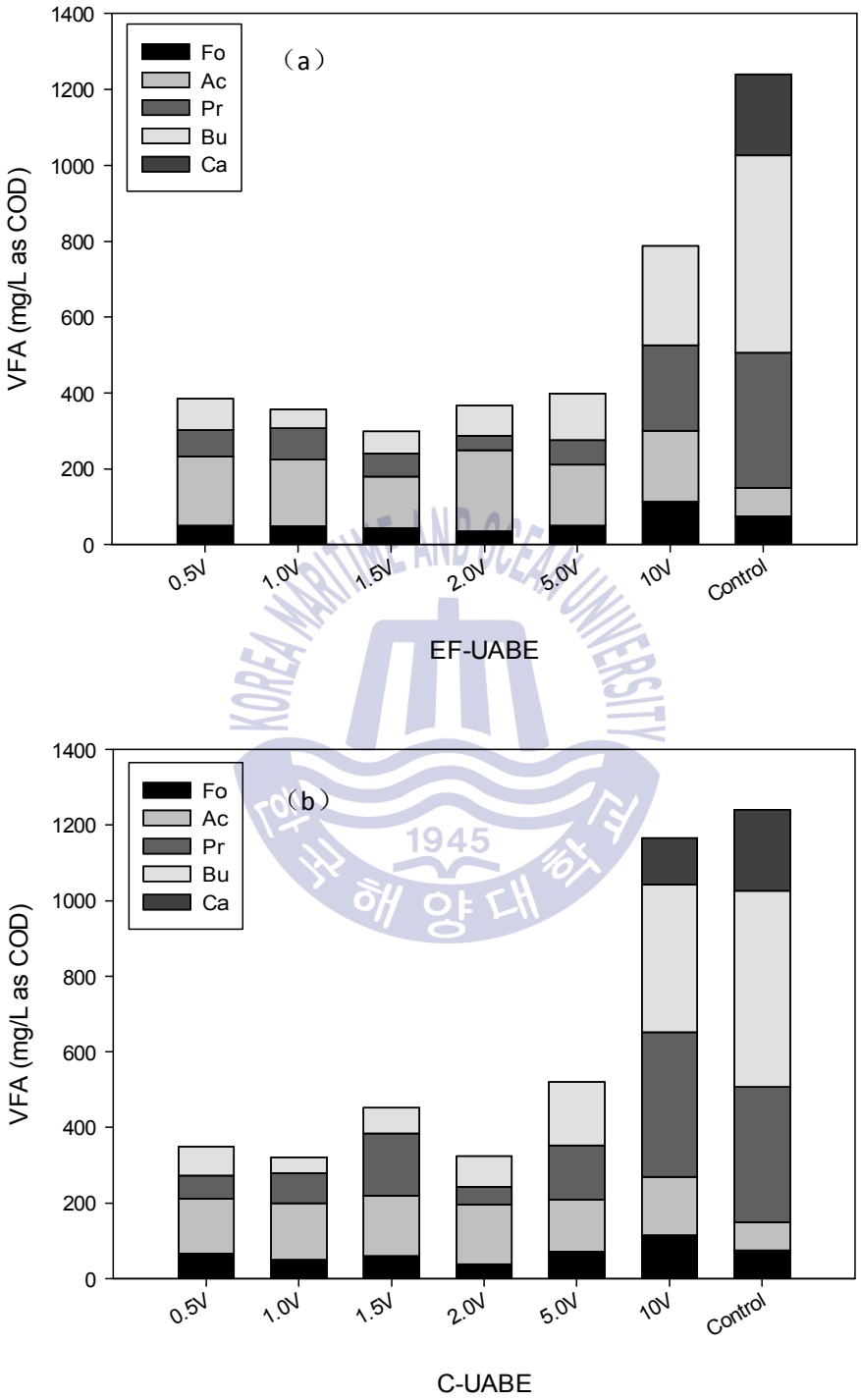


Fig. 4.3. VFA concentrations and their distributions in (a) EF-UABE and (b) C-UABE reactors

4.2 Methane production in the upflow reactors

The control reactor is a kind of upflow anaerobic sludge blanket reactor (UASB), which is one of the popular high-rate anaerobic digestion processes for treating high strength organic wastewater. In the control, the methane production rate was only 101 mL/L.d at steady state (Figure 4.4). The methane production rate in anaerobic digestion depends on the operational conditions such as organic loading rate as well as the type and nature of the substrate (Habeeb et al., 2011; Kaviyarasan, 2014). The low methane production rate in the control is due to the low organic loading rate of 2 g COD/L.d. However, the methane production rate in the EF-UABE and C-UABE reactor was significantly dependent on the applied voltage. When the voltage of 0.5 V was applied to the EF-UABE, the methane production rate was 386 mL/L·d, which was considerably higher than the control. As the applied voltage was increased step-by-step, the methane production rate was more increased in the EF-UABE. The maximum methane production rate in the EF-UABE was 456 mL/L·d, which was obtained at 5.0V. However, the methane production was slightly 394 mL/L·d at 10 V of the applied voltage. It seems that when the applied voltage was 10 V in the EF-UABE, the intensity of electric field was too strong to maintain the activity of electroactive microorganisms.

In the case of the C-UABE, the methane production rate was 399 mL/L·d when the applied voltage was 0.5V. However, the maximum methane production rate was 432.9 mL/L·d, which was obtained at 1.5 V, and at higher voltage than 1.5 V, the methane production rate began to decline. At 5V, the methane production in the C-UABE reactor was 372.8 mL/L·d, which was significantly less than the EF-UABE. When the voltage was 10V, the methane production was only 292.6 mL/L·d in the C-UABE reactor. It seems that an extraordinary high voltage electrolyzes lots of water molecules, and the electroactive microbial communities were possibly disturbed by the hydrogen and the oxygen as the hydrolyzed products.

Table 4.2 Performance of the EF-UABE, C-UABE and control reactors for distillery wastewater treatment

Reactors	Applied voltage (V)	Methane production rate (mL/L.d)	Methane content (%)
EF-UABE	0.5	386.8±14.2	70.3±2.3
	1.0	428.6±23.3	74.4±1.2
	1.5	433.9±17.5	73.4±0.6
	2.0	433.6±19.3	77.1±1.1
	5.0	456.4±19.0	82.6±2.3
	10	393.9±22.1	75.3±6.4
	5.0re	473.9±16.3	82.9±3.3
C-UABE	0.5	399.6±23.5	70.5±3.3
	1.0	429.6±25.9	77.5±2.8
	1.5	432.9±23.5	77.8±2.0
	2.0	382.7±19.5	74.6±2.2
	5.0	372.8±24.4	73.7±2.9
	10	292.6±16.4	60.7±4.3
	5.0re	387.8±17.1	73.9±2.4
Control	\	101.6±22.3	46.1±1.8

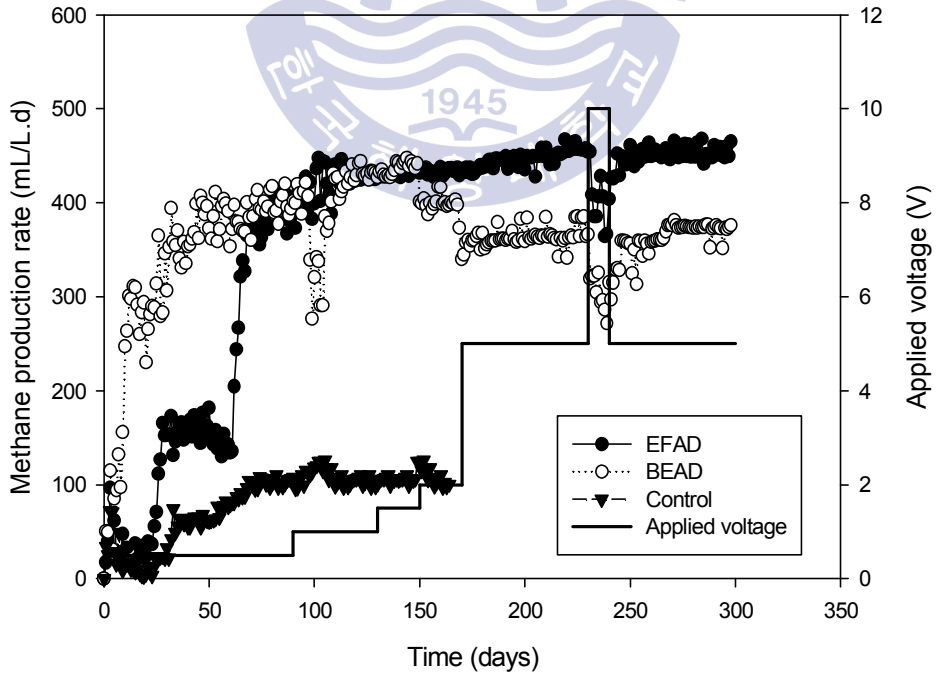


Fig. 4.4. Specific methane production rate in the upflow reactors

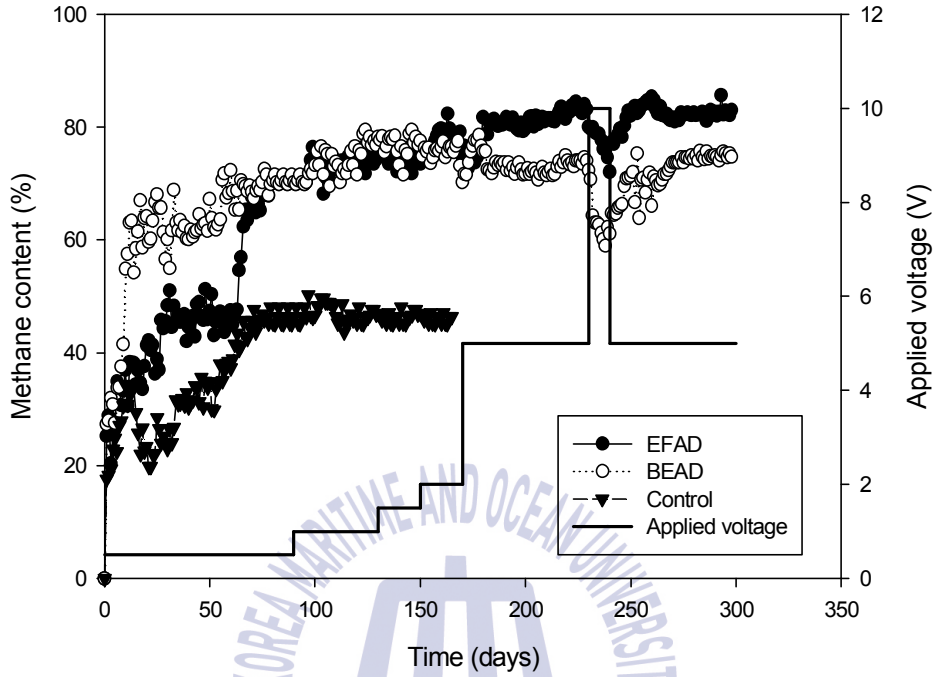


Fig. 4.5. Methane content in the biogas in the upflow reactors

4.3 Organic matter removal in the upflow anaerobic reactors

It is important to confirm whether the organic matter in the distillery wastewater was converted into methane. Fig. 4.6 shows the COD removal in the upflow reactors. For the EF-UABE reactor, the COD removal efficiency was stabilized at 77.2% at 0.5 V of the applied voltage, and then increased to 86.4% when the applied voltage increased to 5.0 V (Table 2). However, when the voltage is increased to 10.0 V, the COD removal efficiency is 85.7%. This is in agreement with the change of methane production in the EF-UABE reactor depending on different applied voltages. However, the COD removal efficiency was only 56.5% in the control reactor. As mentioned above, more methane production in the EF-UABE reactor was attributed to the DIET pathway through the enrichment of electroactive bacteria, which means there are more substrate was oxidized and provide more electrons transferred to produce methane.

Table 4.3 Organic removal efficiency of the EF-UABE, C-UABE and control reactors for distillery wastewater treatment

Reactors	Applied voltage (V)	TCOD removal (%)	SCOD removal (%)	TSS removal (%)	VSS removal (%)
EF-UABE	0.5	77.19±2.1	80.95±1.3	85.44±1.2	86.45±1.1
	1.0	77.60±1.8	90.62±0.8	85.09±1.6	84.76±1.6
	1.5	78.25±1.3	92.65±1.3	83.78±1.4	85.38±0.9
	2.0	78.88±1.2	91.99±1.8	83.74±1.3	86.82±1.4
	5.0	86.38±1.7	93.19±1.4	81.86±1.8	85.32±1.8
	10	85.69±2.8	90.15±3.1	80.85±2.6	85.17±2.0
	5.0re	88.35±1.2	94.35±1.3	83.60±1.1	87.87±1.6
C-UABE	0.5	76.17±2.8	81.60±1.8	83.55±1.1	85.89±1.3
	1.0	75.21±3.1	88.36±1.7	84.65±1.3	83.16±1.2
	1.5	75.97±2.7	89.31±1.4	84.01±1.5	86.15±1.1
	2.0	73.01±1.9	85.33±1.0	83.30±1.2	83.46±1.6
	5.0	69.41±1.4	78.75±1.6	58.47±1.8	65.32±1.8
	10	65.49±2.9	62.65±2.4	53.19±2.6	59.32±2.2
	5.0re	69.19±1.8	76.04±1.9	59.58±1.3	63.88±1.3
Control	\	56.47±1.5	71.40±1.3	37.19±1.1	57.80±1.2

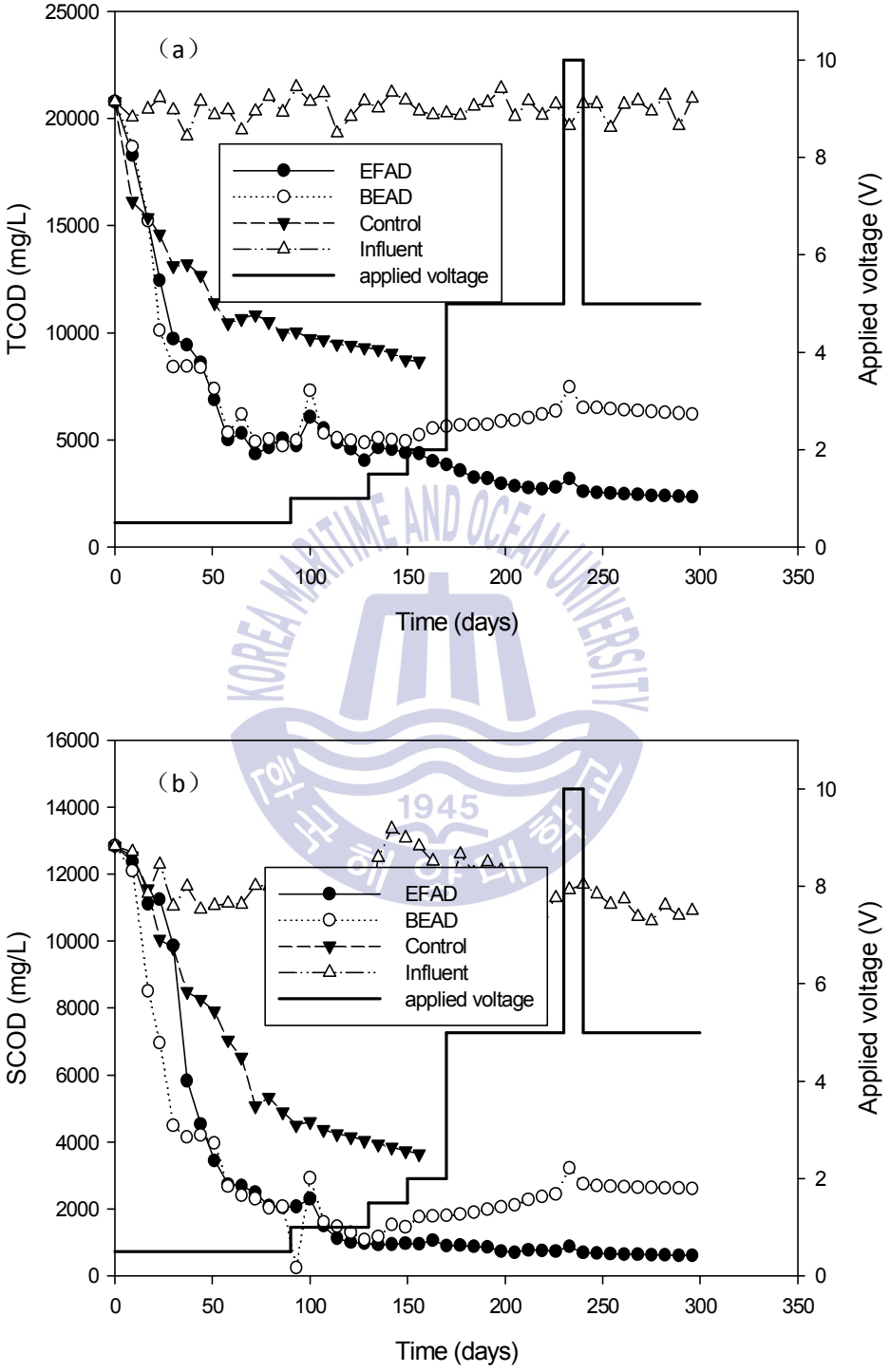


Fig. 4.6. Changes in effluent of (a) TCOD and (b) SCOD in the upflow reactors

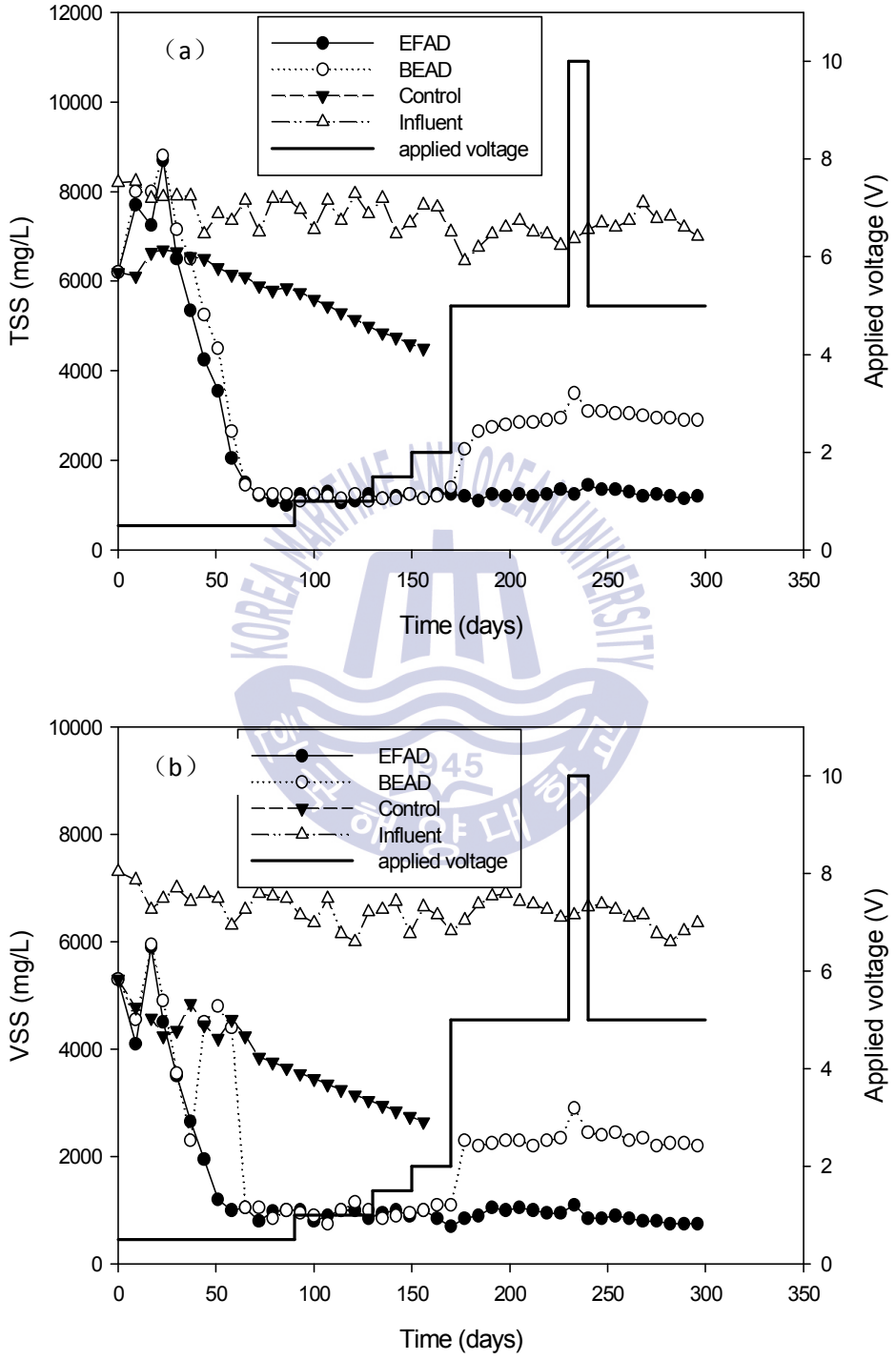


Fig. 4.7. Changes in effluent of (a) TSS and (b) VSS in the upflow reactors

4.4 Electron transport pathway for methane production

The electron transfer efficiency from substrate to methane in the anaerobic digestion significantly varies depending on the electron transport pathway. The concentration of intermediates and their distributions that depends on the ratio of $NADH/NAD^+$ give the useful information on the electron transfer pathway (Feng et al., 2016a; Lyberatos & Skiadas, 1999; Ren et al., 2002). In this study, the VFA of the control was as high as 1,240 mg COD/L, and the formate was 75 mg COD/L, and caproate was 213 mg COD/L (Fig. 4.3). In conventional anaerobic digestion, the IIET via the intermediates, such as acetate, hydrogen and formate, is the main pathway for the electron transfer from substrate to methane. However, it has recently been reported that electroactive microorganisms, such as *Geobacter metallireducens* and *Geobacter sulfurreducens,* can directly transfer electrons generated from the oxidation of acetic acid to electrotrophic methanogens, such as *Methanosaeta* and *Methanosarcina*, through the outer membrane c-type cytochrome or conductive pilus-like structure in bioelectrochemical reactors. This is called as biological direct interspecies electron transfer (bDIET) (Kouzuma et al., 2015; Shen et al., 2016; Zhao et al., 2016). The bDIET for methane production has also been observed to occur in anaerobic granules in upflow anaerobic sludge blanket reactor (Kouzuma et al., 2015; Shen et al., 2016; Zhao et al., 2016). Generally, the bDIET is more favorable thermodynamically than the IIET for the energy transfer (Dubé & Guiot, 2015 Shen et al., 2016). However, depending on the environment and operating conditions, including pH, temperature, substrate type, and organic and liquid loading rates, the electron transfer pathway for methane production may vary. In the control, high concentrations of formate, propionate, and caproate indicate that the IIET pathway for formic acid production was possibly inhibited by the low pH value of the acidic feed distillation wastewater.

At 5.0 V of the applied voltage, the total VFAs for the EF-UABE and C-UABE reactor were 298 mg COD/L and 787 mg COD/L, respectively, which were lower than 1,240 mg COD/L in the control. The main components of VFA in the EF-UABE and C-UABE reactor were acetate, propionate and butyrate, but caproate was not detected. However, the formate concentrations of the EF-UABE and C-UABE reactor were only 43 mg COD/L, which was much lower than 75 mg COD/L of the control reactor. Electroactive microorganisms can be readily enriched in bioelectrochemical reactors equipped with anodes and cathodes (Zhao et al., 2016). In previous studies, the increase in the combination of methane production with anaerobic digestion and bioelectrochemical devices was usually described by

bioelectrochemical reduction of carbon dioxide to methane at the cathode surface (Villano et al., 2016; Wang et al., 2009; Zhao et al. 2014). The methane produced by the reduction of carbon dioxide on the cathode surface is proportional to the faradaic current flowing between the cathode and the anode. However, the current that monitored in the external circuit was too small to describe all of the methane production. It seems that there are other important electron transport pathways in the C-UABE reactor for methane production rather than direct interspecies electron transfer via electrodes (Feng et al., 2016a; Song et al., 2016). During anaerobic digestion, electroactive microorganisms can transfer electrons directly to methanogenic bacteria through conductive materials (such as activated carbon, graphite particles, and magnetite) (Kouzuma et al., 2015; Shen et al., 2016). However, in this study, conductive materials in acidic wastewater are unlikely to be present.

In the bulk solution for the EF-UABE and C-UABE reactor, the cyclic voltammogram (CV) was obtained. In the EF-UABE reactor, the peak potentials currents were xx mV for the oxidation and xx mV for the reduction when the voltage of 0.5 V was applied (Fig. 4.8). The peak potentials and the peak currents were dependent on the applied voltage. The highest value of the peak current was obtained at 0.5 V of the applied voltage, which was consistent with the methane production rate. The redox peak currents represent the electrochemical activity of electroactive microorganisms, as well as the redox substances in the bulk solution [11, 31, 32]. These electrochemical data in the CV indicates that the electron transfer for methane production at 5.0 V of applied voltage is more favorable than the 0.5 V. Based on the CV data, the potential electron transfer pathways for methane production in the bulk solution include bDIET, sIIET and the iIIET via hydrogen and formate. In the EF-UABE reactor, the methane yield was significantly higher than in the control reactor, which indicates that the bDIET pathway was enhanced. It is known that the bDIET pathway for methane production is activated between the electroactive species including the EFB and EMM [3, 12, 17, 18]. Acetoclastic methanogenic bacteria such as *Methanosaeta* and *Methanosarcina* belong to the EMM group. In the C-UABE, the contribution of bDIET for the methane production was confirmed from the potentials and the currents of redox peak in the CV data (Fig.4.9).

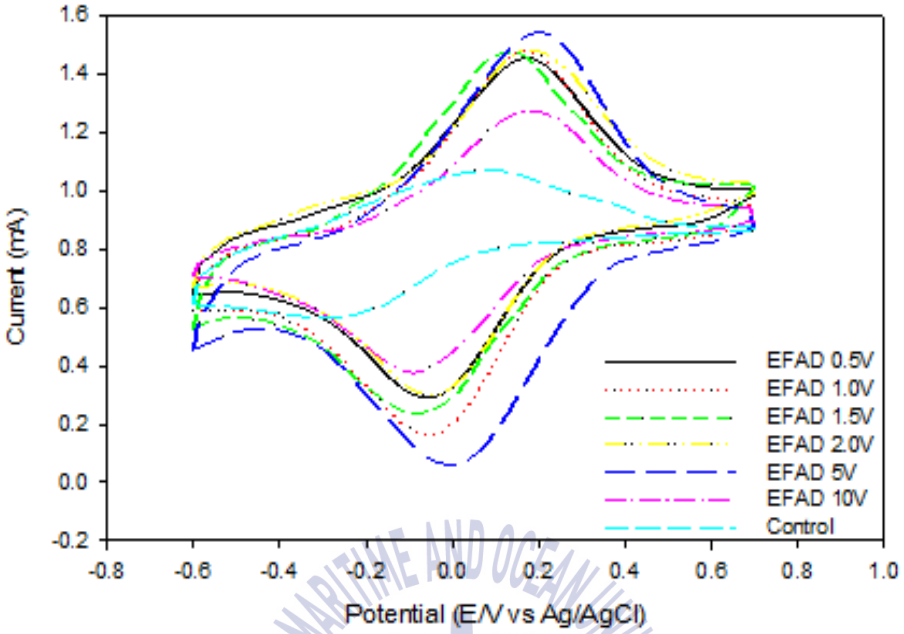


Fig. 4.8. Cyclic voltammogram of bulk liquid in EF-UABE and control reactors

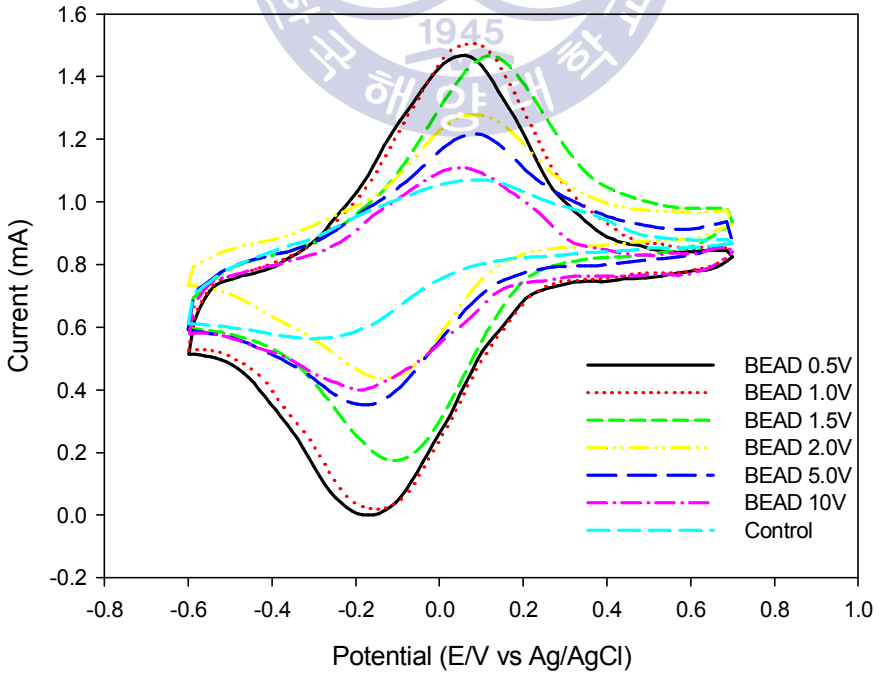


Fig. 4.9. Cyclic voltammogram of bulk liquid in C-UABE and control reactors

Chapter 5： Conclusion

1. The methane production rate from distillery wastewater is significantly improved in the upflow anaerobic bioelectrochemical reactor than the upflow anaerobic reactor without the electrode.

2. The methane production rate in the upflow anaerobic bioelectrochemical reactor using electric field increases to 456.4 mL/L.d as the applied voltage increases up to 5.0 V. However, the maximum applied voltage was 1.5 V in the conventional upflow anaerobic bioelectrochemical reactor and the methane production was 432.9 mL/L.d.

3. The direct interspecies electron transfer for methane production in the bulk solution in the upflow anaerobic bioelectrochemical reactor was enhanced by the electric field, as well as the polarized bioelectrode.

4. The bioelectrochemical anaerobic digestion with the insulated electrode is a feasible technology in the field for the stabilization and methane recovery from high strength organic wastewater.

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